

RESPONSE OF A LIVING ORGANISM, UNDER "CONSTANT
CONDITIONS" INCLUDING PRESSURE, TO A BARO-
METRIC-PRESSURE-CORRELATED, CYCLIC,
EXTERNAL VARIABLE¹

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Many overt biological rhythms are known which exhibit cycles of 24 hours with such great precision, at least statistically, that the phases of the cycles do not become significantly altered with respect to external day-night even after weeks or months in conditions of constant darkness and temperature. Such rhythms have been reported in a wide variety of organisms and for many processes (see reviews by Welsh, 1938; Kleitman, 1949; Webb, 1950; Caspers, 1951). Less commonly known is the fact that persistent cycles of lunar-day frequency appear also widespread (*e.g.*, Gompel, 1937; Brown, Fingerman, Sandeen and Webb, 1953; Rao, 1954; and Ralph, 1956). One of the most striking properties of these rhythms appears to be the temperature-independence of their frequency (Brown and Webb, 1948; Brown, Webb, Bennett and Sandeen, 1954; Pittendrigh, 1954). To account for these rhythms, either 1) there must be an astonishingly precise, temperature-independent clock-mechanism present within the organisms, or, 2) they are receiving signals with the same average frequencies from external sources and which serve in some manner as pacemakers, or, 3) some combination of these two. Fluctuations in cycle-length from day to day under constant conditions in such regular overt cycles as running in the mouse (Johnson, 1939), and crab color change (Brown, Webb and Bennett, 1955), despite the great precision of the average lengths of the cycles, suggest strongly the operation of an external pacemaker. Proof for the existence of at least a reasonably precise internal clock has been advanced (Brown, Webb and Bennett, 1955; Renner, 1955) through studying the results of rapid east-west geographic displacement of animals, but it is still not demonstrated that this is sufficiently precise to serve as the exclusive mechanism. Since there are known 24-hour physical cycles which appear to operate on universal time (*e.g.*, atmospheric electrical potential), it may be argued that there is still lacking definitive proof that regular pacemakers are inoperative even during such rapid longitudinal displacement of organisms.

In the course of seeking possible effective external rhythmic signals of the appropriate frequencies, average rhythms of O₂-consumption of primary solar and primary lunar frequencies have been found in all organisms so far examined for them. These have included organisms as widely diverse as seaweed, snails, crabs, *Triturus*, worms, carrots and potatoes (Brown, Bennett and Webb, 1954; Brown, Webb, Bennett and Sandeen, 1955; Brown, Freeland and Ralph, 1955). Studies of the

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activities of quahogs, oysters and rats indicate that these, too, possess the same general types of average solar and lunar rhythms (Brown, 1954a; Brown, Shriner and Ralph, 1956; Brown, Bennett, Webb and Ralph, 1956).

The fact that there are known to exist average rhythms of barometric pressure of solar- and lunar-day frequencies led to attempts to correlate barometric pressure changes with the observed biological cycles, and some definite correlations were found not only between the hourly rates of barometric pressure change and of concurrent O₂-consumption, or activity, but also between mean daily levels of pressure and mean daily metabolic rates (Brown, Freeland and Ralph, 1955; Brown, Webb, Bennett and Sandeen, 1955). There were shown, furthermore, to be such distinct similarities between the general forms of the daily pressure changes and daily variations in oyster and quahog activity (Brown, Bennett, Webb and Ralph, 1956) as to suggest strongly more than a fortuitous relationship. This resemblance was sometimes a direct one and other times one of a mirror image. The experiments to be described herein were initially planned to resolve the problem of a possible direct role of barometric pressure variations.

MATERIALS AND METHODS

Potatoes, *Solanum tuberosum*, which were purchased at a local grocery store constituted the organism. With a cork-borer, cylinders 22 mm. in diameter and about 1½ cm. high, each bearing an eye, were cut and the injured surfaces allowed to heal. In the experimental situation of constant temperature and constant very low illumination these developed sprouts. They were replaced during the experiments only after long periods when they grew too large for the respirometer vessels.

The respirometers were of the type designed by Brown (1954b) which permitted continuous automatic recording of O₂-consumption. The instrument was modified, however, in such a manner as to permit four respirometers to record as a unit, and the recorder was small enough to be sealed along with the respirometers in a barostat (Fig. 1). The barostat first successfully used was an 11 × 24-inch vertical autoclave sealed with an O-ring. Since it was not possible to view the interior once this was sealed, a simple barograph was included in the system to assure an absence of leakage. Later, five simplified barostats were constructed. These consisted of ½₃₂-inch copper cylinders, 10¼ inches in diameter and 22 inches deep, with dished bottoms and a 1-inch-wide, ⅛-inch-flat, ground-brass rim. These, covered by twelve-inch vacuum desiccator covers with an electrical inlet for the recorder motor and a glass stopcock passing through a rubber stopper, served as excellent barostats. A ⅜-inch brass tripod ring, fitting snugly within the cylinder served as a platform to support the recorder, with its divers hanging into an enclosed, 10-inch-deep water bath. Each copper cylinder was supported in a 55-gallon steel drum full of water maintained at constant temperature. The temperature settings for the five baths all lay in the range 19.6 to 19.9° C.

The apparatus was shielded from all light except that from a row of 7½-watt, opalescent, incandescent lamps which provided a continuous illumination of about 1–2 ft. c. at the desiccator-cover surface, and obviously some far lower constant illumination at the surface of the organisms.

One cylinder of potato was placed in each of the four respirometers of a recording unit and the whole lowered into a barostat, sealed, and the barostat as-

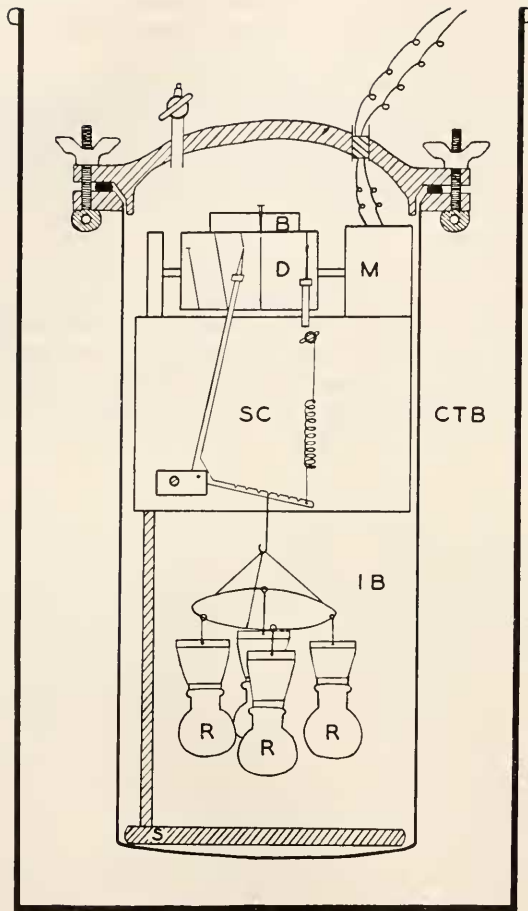


FIGURE 1. Four respirometers (R) hanging into a water bath (IB) from a spring scale (SC) ink recording system registering on a 24-hour drum (D) activated by an electric clock motor (M). A barograph (B) also registers on the same drum. The whole is supported by a stand (S) in a sealed copper-and-brass container which sets deeply in a constant-temperature bath (CTB).

pirated to reduce the internal pressure to 28.00 in Hg. This was sufficiently below the lowest normal external pressure in Evanston, Ill. (ca. 28.5) that the cover would always retain its seal. A barometer in each barostat permitted one to be continuously assured of the absence of leakage. Usually the apparatus was then left undisturbed for three to five days. This was the length of time the recording needle required to spiral down the five-inch-length of the three-inch-diameter brass drum to the recording base-line, with the drum turned one revolution per day with an electric time-clock mechanism. The paper was a smooth-surface, bond, writing paper held in place with rubber cement. The pens were small L-shaped glass capillaries with polished tips and contained a 50–50 glycerine-water mixture, colored with

neutral red. In more recent work, millimeter graph paper and commercially available thermograph pens (counterbalanced) and ink have been found simpler to use.

The springs used were wound from 0.014-inch, 8-18, stainless-steel wire, and were 5 cm. long and about $\frac{1}{2}$ cm. in diameter. Over the recording range (10 gms./cu.) in this mechanical recording system, there was only about a 10% variation from linearity from the first to the last day of recording. There was, undoubtedly, also, a very minor, gradual reduction in pressure in the barostat over a recording period as the O_2 -reservoirs of the respirometers were depleted, but this was not significant in view of the large size of the ratio: barostat gaseous volume/ O_2 -reservoir volume.

Respirometers were always loaded sometime between 8 A.M. and 8 P.M. In the analyses to follow, except when specifically stated otherwise, no data were used before the first midnight after the sealing of a barostat, nor of the day they were opened. Hence, there were usually complete, undisturbed, calendar days of data. All times are central standard.

From April 1 through April 26, 1955, only one barostat was in operation. From April 28 through June 8, five were in continuous operation.

RESULTS

It was apparent even after the first day of recording that the rate of O_2 -consumption in the rat is by no means uniform even under constant temperature, illumination, oxygen, CO_2 , humidity and pressure. There were fluctuations usually with more than one conspicuous maximum a day. Eight sample days picked at random from the data, illustrating the variation in the two to five separate barostats in which recordings were complete on that particular day, are illustrated in Figure 2. These

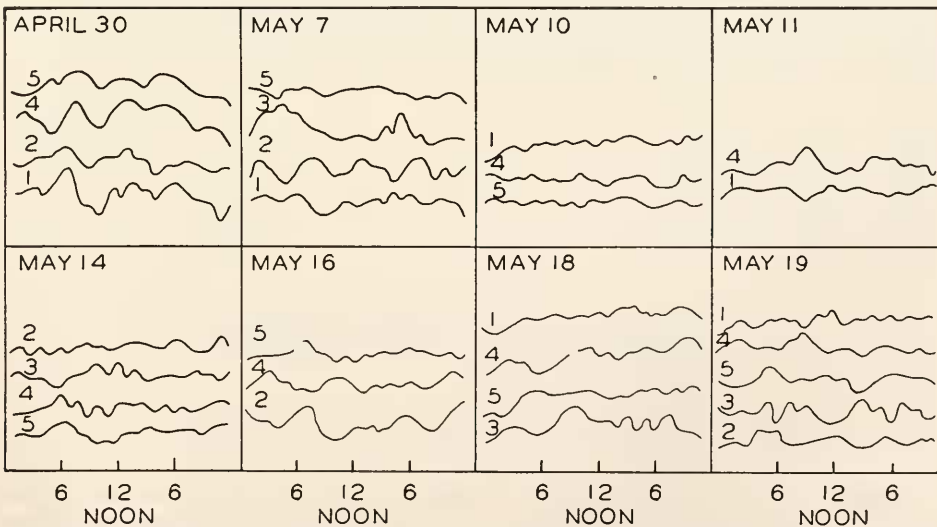


FIGURE 2. The forms of the daily fluctuations in oxygen consumption (three-hour sliding averages) measured on eight randomly selected days in all those (1 to 5) barostats from which records were complete for that day. The ordinate scales are comparable for all, but the patterns have been separated for clarity.

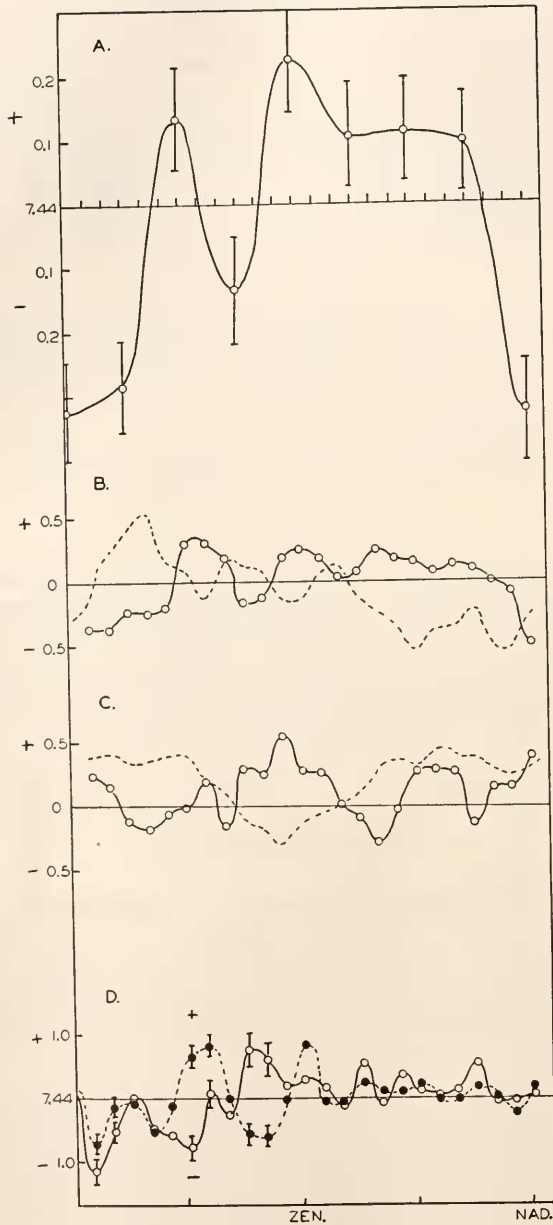


FIGURE 3. A. The mean rates of O_2 -consumption for the 8 three-hour periods of the solar-day for all the data of the 154 barostat-days. These are expressed as deviations from the daily means.

B. Moving three-hour means of the daily variation for 100 randomly selected days in 1955 (solid line), compared with the comparable mean daily cycle obtained at the same time of year in 1954.

TABLE I

The percentage increase from lowest to highest values of the daily cycles illustrated in Figure 2

Unit	Date							
	Apr. 30	May 7	May 10	May 11	May 14	May 16	May 18	May 19
1	133	38	71	33			73	49
2	37	73	26		35	84		31
3		67			35		77	52
4	100			46	38	34	89	54
5	67	23	10		80	45	72	32
Av.	82	50	35	40	47	54	78	45

are three-hour moving means. Table I includes the percentage increase from lowest to highest values of the days illustrated. One hundred and fifty-four complete barostat-days of data, including the 29 illustrated in Figure 2, were obtained, together with numerous fractional barostat-days.

Inspection of the daily patterns revealed that there were clearly as many detailed patterns as there were days of data. But there appeared to be a suggestive generic similarity in the records obtained for any given day in entirely independent barostats with respect to amplitude of the fluctuations, gross trends, and approximate times of the major maxima and minima. There seemed, apparently too commonly to be attributed simply to chance, a tendency for one or more of the records to show on any given day an inversion of some of, or even the greater part of, the daily pattern relative to the other concurrent ones. In nearly every instance, however, if one obtained the average daily pattern for a three- to five-day period of continuous recording in one barostat, there was a distinct maximum about 6 or 7 A.M. and a minimum about 9 or 10 A.M.; also, low values nearly always characterized the early morning hours.

In Figure 3A is plotted the mean daily cycle for all barostat-days of data. This is plotted as the mean differences from the mean hourly rate for the whole day for each of the eight three-hour periods of the day. The mean daily fluctuation is seen to be about 8%. The mean hourly value for all these data was 7.44 with the range extending from about 5 to 14. For each value in the figure is indicated the standard error of the mean.

The mean daily pattern is in large measure a mirror image of the one obtained without the use of a barostat during the 29-day period, May 12–June 9, 1954 (Brown, Freeland and Ralph, 1955) involving both inversion of the major 24-hour cycle and the secondary fluctuations superimposed on the larger cycle. Moving three-hour averages of 100 randomly picked days in 1955 and the 1954 cycle are seen in Figure 3B. In 1955 the highest rates for the day occurred in the afternoon; in 1954 the highest rates were found in the morning.

C. The mean lunar day cycle for the potato for the month of May, 1955 (solid line), compared with that obtained in May, 1954 (broken line).

D. The mean daily cycles with sample standard deviations of the means, for the 99 positive barostat-days of O₂-consumption, and the 55 negative ones.

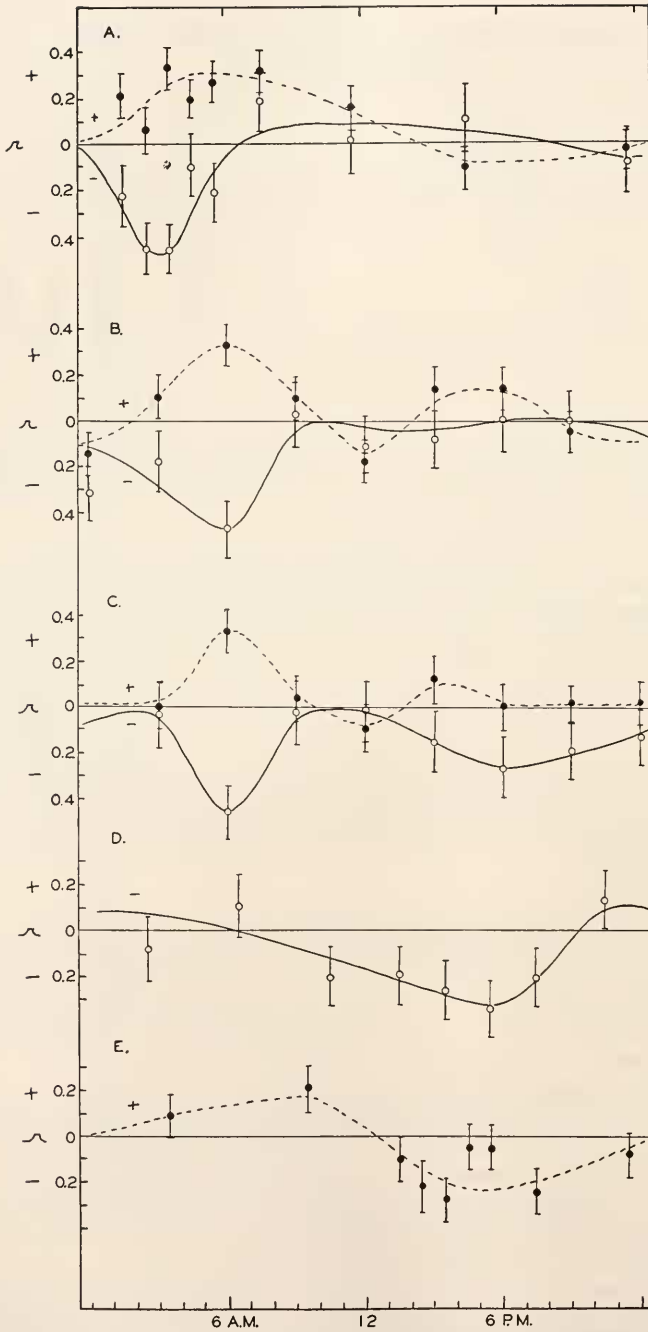


FIGURE 4. A. The correlations between the rate of O_2 -consumption during the 4 to 7 A.M. period of a day and the mean rate and direction of barometric pressure change for the same day

There was also an apparent mean lunar-day cycle in the potatoes. It, too, showed a general major inversion relative to the comparable lunar-day cycle obtained in 1954. When only 29 consecutive days of data in May were used, in order exactly to randomize a daily cycle and hence to obtain the minimally distorted form of a lunar-day cycle, the results seen in Figure 3C were obtained. In the same figure is the form of the lunar-day cycle obtained in 1954 without the use of a barostat. These are both three-hour moving means.

Brown, Freeland and Ralph (1955) have shown that for the potato in respirometers subjected to normal fluctuations in pressure in 1954, there was present, even after all appropriate corrections for pressure changes had been made, a correlation coefficient of -0.58 ± 0.035 between the hourly values of O_2 -consumption and the concurrent rates and direction of barometric pressure change over a month. That this demonstrated a direct response to some external factor fluctuating with barometric pressure changes was ascertained by obtaining the coefficient for the same monthly period using metabolism on the hours of day n and the pressure changes for day $n + 1$. Now, the value dropped to 0.217 ± 0.038 , a value not significantly different from the auto-correlation of hourly pressure changes on two consecutive days. For reasons discussed in that paper it was considered unlikely that the response was a direct one to pressure on the part of the plant. This last presumption is supported amply by this work in which the pressure was kept constant during the recordings.

Although the earlier work had shown a correlation between the hourly rates of O_2 -consumption in the potato and the concurrent rate and direction of barometric-pressure change in organisms subjected to barometric-pressure fluctuation, using data available in the current experiments, no correlation with the hourly pressure change in the external environment was found. With 2374 hours of data, a value of $r, 0.020 \pm 0.021$ was obtained.

An attempt was made to test an hypothesis that there was not a correlation with the barometric pressure changes in these data obtained in 1955 because of abrupt 180° -shifts of the phases of at least one important component of daily rhythmicity in the plants. The 154 barostat-days of daily patterns of respiration were, by inspection, divided into two categories on the basis of whether a maximum and minimum occurred about 6 and 9 A.M., respectively, or the cycle at these hours was ap-

and the two preceding days for various two-hour periods of the day, for both the positive and negative groups of potatoes.

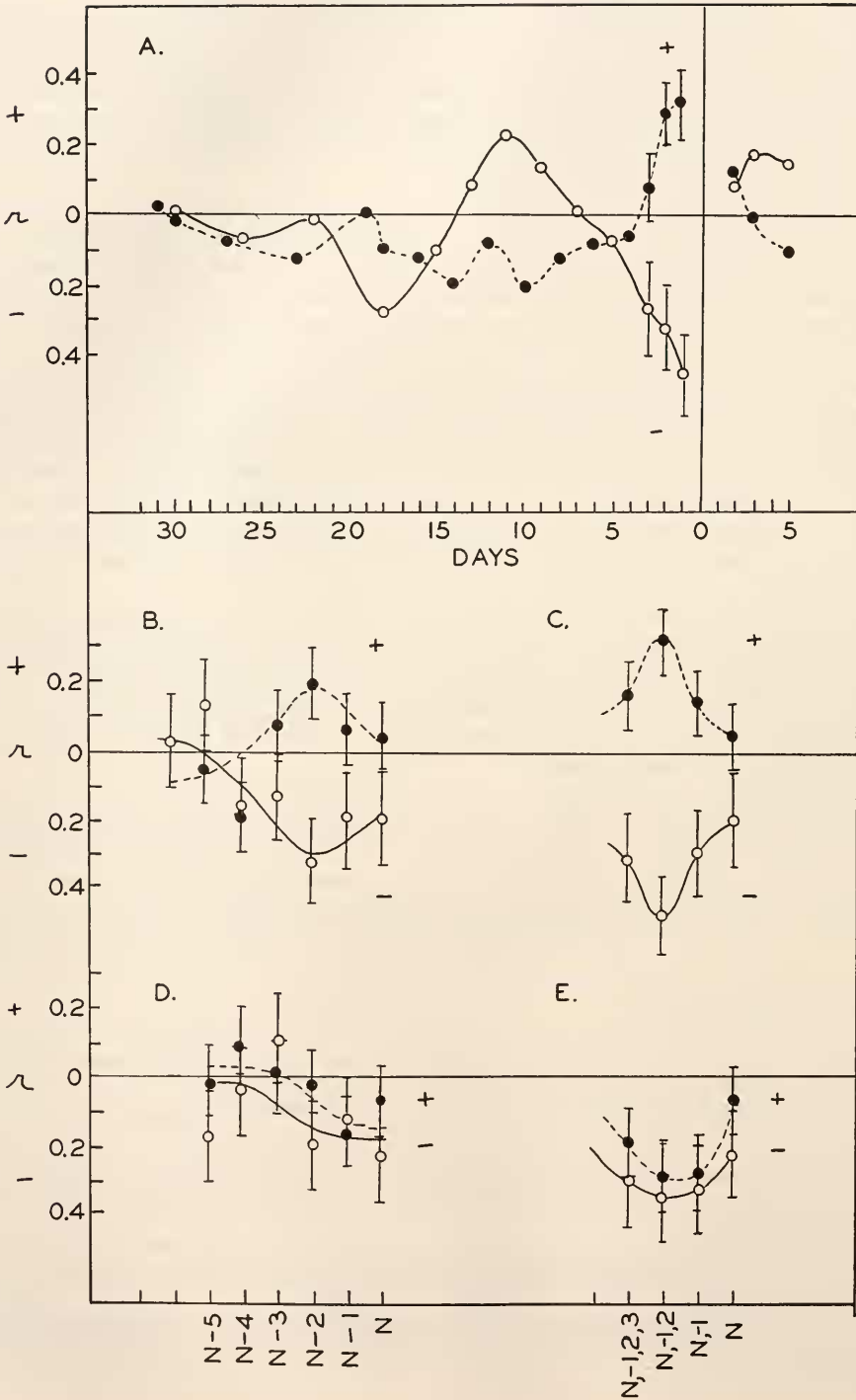
B. The correlations between the mean rate and direction of barometric pressure change during the 2 to 4 A.M. period of a three-day period and the mean rate of O_2 -consumption for various three-hour periods on the last of the three days.

C. The correlations between the mean rates of O_2 -consumption during various three-hour periods of the day and the mean three-day changes in barometric pressure for the two preceding hours of the day.

D. The correlations between the mean rate of O_2 -consumption during the 4 to 7 P.M. period and the mean, three-day changes in barometric pressure during various two-hour times of day, for the negative group.

E. The correlations between the mean rate of O_2 -consumption during the period 5 to 8 P.M. and the mean three-day change in barometric pressure for various two-hour periods of the day for the positive group.

The potatoes were maintained in barostats throughout the study. The values of O_2 -consumption used are deviations from the daily mean in order to eliminate long-period trends in metabolic rate.



parently inverted, with a minimum about 6 and a maximum about 9 A.M. The latter were termed the negative group since in the form of the minor fluctuations this was more nearly the form of the 1954 patterns which showed the negative correlation with concurrent barometric pressure changes; the former was termed the positive group.

Barostat No. 1 yielded 14 negative days and 33 positive ones; No. 2 gave 7 negative and 23 positive; No. 3 showed 17 negative and 10 positive; No. 4 had 11 negative and 10 positive; and No. 5 provided 6 negative and 23 positive. Though both positive and negative responses could be found in different barostats on a single calendar-day, there were two or three periods of three or four days, when there seemed to be a high percentage of negative cycles.

In Figure 3D are seen the mean daily patterns for the 99 positive and 55 negative days expressed as mean hourly deviations from the daily means. It is very interesting to note that though these were based exclusively upon selection of an apparent inversion between about 4 and 11 A.M. between the two groups, the remainder of the daily patterns are in extraordinary agreement for the two groups even to most of the minor fluctuations. It was now assumed that, at least for the 4-11 A.M. period, the potatoes were divided into two separate, relatively homogeneous populations with respect to any sign of correlation with any external pressure changes which might be present. On the other hand, it is very important to emphasize here that there was no *a priori* reason why a single value for either of these two groups for any arbitrarily selected single time of day should show any correlation whatsoever with external atmospheric barometric pressure changes.

First, an intensive search was made to learn whether the average rate of O₂-consumption in the 55 days for potatoes of the *negative* group for the 4-7 A.M. period was correlated with external pressure changes in any manner. It was discovered that a correlation, -0.46 ± 0.106 , existed between the total respiration at this time on day n , expressed as deviation from the daily mean, and the algebraic sum of the rates of the pressure changes at 2-4 A.M. on days n , $+(n-1)$, $+(n-2)$. In Figure 4A it is readily seen that there is a rapid drop to no correlation as one moves away from 2-4 A.M. pressure changes in either direction. In Figure 4A it is also seen that not only did the 99 days of the positive group also show a significant correlation for essentially the same kind of relationship, but with opposite sign, 0.326 ± 0.089 . There was similarly a loss of correlation with pressure changes as one moved to other times of day.

FIGURE 5. A. The correlations between the mean deviations in rate of O₂-consumption during the 4 to 7 A.M. period and the mean change in barometric pressure during the 2 to 4 A.M. period during various three-day periods centering on days ranging from 31 days earlier to 5 days later than the day of correlated O₂-consumption, for both positive and negative groups.

B. The correlation between the rate of O₂-consumption during the 4 to 7 A.M. period on day n with the changes in barometric pressure during the 2 to 4 A.M. period on single days ranging from day n to day $n-6$.

C. Correlations between O₂-consumption and barometric pressure for the same hours as in B of the solar day, but now with the means of pressure including increasing numbers of earlier days up to three.

D and E. The same as B and C except that the correlations of pressures at 2 to 4 P.M. with O₂-consumption at 5 to 8 P.M. are used for the positive group and barometric pressure changes between 4 and 6 P.M. are correlated with O₂-consumption for the 4 to 7 P.M. period for the negative group.

Figure 5 demonstrates in two ways that the correlations that have been described are actually the result of a summation by the potato of three or more days of fluctuation in an external factor. First, for both the positive and the negative groups, the correlation not only increases to a maximum as one increases the number of days up to three, but there is a reduction beyond that point (Fig. 5C). Second, it is seen that the best single-day pressure change correlation occurs for both groups with pressure change on day $n - 2$, with lower or no correlation either earlier or later (Fig. 5B). In Figure 5A it is seen that there is a rapid reduction in correlation with summed three-day pressure changes for both positive and negative groups as one attempts the correlations with earlier days or later. The apparent drifts in correlations suggested for three-day periods earlier than $n - 2$, $n - 3$, and $n - 4$, and extending for nearly three weeks probably reflect only auto-correlations of pressures. However, only the correlation with $(n) + (n - 1) + (n - 2)$ is significantly different from 0.

Figure 4B demonstrates that the three-day sums of pressure changes at 2-4 A.M. (days n , $n - 1$, $n - 2$) are not significantly correlated with the rates of respiration (day n) for any time of the day other than the 4-7 A.M. period for either the positive or negative groups. In Figure 4C the rates of O_2 -consumption at various periods of day n are correlated with the sums of three-day (day n , $n - 1$ and $n - 2$) pressure changes for the two-hour earlier period. There is a suggestion herein that there may be a second period in the day, in the afternoon, when there is a real correlation for the negative group, though a similar suggestion is lacking for the positive one. When this possibility was explored in detail for the negative group (Fig. 4D), the highest correlation was found between the 4-7 P.M. deviation in O_2 -consumption and the 4-6 P.M. (day n , $n - 1$, and $n - 2$) pressure change, -0.347 ± 0.12 . There was also found an afternoon correlation for the positive group but not with the same temporal relationship (Fig. 4E). The highest correlation was found between the 2-4 P.M. pressure change and the 5-8 P.M. deviation in respiration, -0.273 ± 0.091 . Just as the forms of the mean daily cycles for both groups of potatoes for this part of the days were quite similar, the correlations here also bore the same sign.

It is seen from Figure 5E that the correlation in the afternoon is maximal with the summation of three days (n , $n - 1$, $n - 2$) of pressure changes, with a reduction with fewer or greater number of days. The correlations with single days (Fig. 5D) are relatively small, being about equal for days n , $n - 1$, and $n - 2$.

Evidence supporting the earlier assumption of there being a tendency of the potatoes actually to be reversing from time to time the sign of their response to some external factor became apparent from a study of structure in correlation scatterplots. One of these was the following. When one made no selection whatsoever of the 154 barostat-days of data and determined only the relationship between the extent of the fluctuation, *positive or negative*, from the daily mean for the 5-7 A.M. O_2 -consumption on day n , and the algebraic sum of the barometric pressure changes from 2 to 6 A.M. for days n , $n - 1$, and $n - 2$, a coefficient of 0.361 ± 0.070 was obtained. No correlation was obtained unless the sign of the response was ignored.²

² Further support of this hypothesis of inversions has been obtained since the preparation of this report. With 960 complete potato-days, including hourly data, obtained in barostats during October and November, 1956, the correlation between $3 \times 3 \times 3$ -day moving means of the 2 to 6 A.M. barometric pressure change on day n and the three-day moving mean of the 6 A.M. devia-

This highly significant result clearly justified the earlier division of the data into positive and negative cycles. Again, ignoring the sign of deviation of O_2 -consumption from daily mean, the correlation between the three-day ($n, n-1, n-2$) 5-9 A.M. barometric pressure change and O_2 -consumption at 8-11 A.M. yielded a lower value of 0.223 ± 0.077 .

There was, further, with no selection of the 154 barostat days of data, found to be a correlation, 0.305 ± 0.073 , between the algebraic sum of the 2-6 P.M. barometric changes of day $n, n-1$, and $n-2$ and the deviation in O_2 -consumption of the potatoes from the daily mean at the 4-7 P.M. period, if one used simply average rate of pressure change, irrespective of sign. It was evident, however, from examination of a scatterplot that the correlation for those 14 atypical days on which the pressure showed an overall rise at this time of day during the three-day period, was distinctly inferior to that for the 140 days of data of periods for which the pressure was (as typically) falling. The coefficient of correlation between the mean three-day rate of fall in afternoon barometric pressure and 4-7 P.M. deviation in rate of O_2 -consumption was 0.355 ± 0.0734 .

TABLE II

Correlation between rate of change of barometric pressure and O_2 -consumption

Bar. pressure	Potato	Correlation
11-3 A.M.	2-4 A.M.	-0.087 ± 0.08
2-6 A.M.	5-7 A.M.	$\pm 0.361 \pm 0.07$
5-9 A.M.	8-11 A.M.	$\pm 0.223 \pm 0.077$
8-12 A.M.	11-1 noon	$+0.024 \pm 0.08$
11-3 P.M.	2-4 P.M.	-0.056 ± 0.08
2-6 P.M.	4-7 P.M.	-0.305 ± 0.073
5-9 P.M.	8-10 P.M.	-0.084 ± 0.08
8-12 P.M.	11-1 midnight	-0.194 ± 0.078

No other real correlations (except possibly for the 8-12 P.M. pressure change and midnight (11 P.M.-1 A.M.) O_2 -consumption) could be found for the potatoes as a whole over the day, even when the structure of correlations was examined for possible reversing responses. The foregoing results are included with some additional ones in Table II.

The potatoes of barostat No. 3 for the 560 hours obtained during May and June showed an hourly correlation of their deviations from the daily means with the concurrent deviations from the daily means of barometric pressure, of $+0.238 \pm 0.040$, a value highly significantly different from zero. That this was a correlation with the concurrent hourly values and not based simply upon similarities of the mean forms of two independent average cycles was readily apparent by finding no correlation for the same period with the pressures of day $n+3$ (0.0923 ± 0.0451), day $n-1$ (0.100 ± 0.0425), and for half the period with day $n+1$ (0.0118 ± 0.059), day

tions from the daily means of $5 \times 3 \times 3$ -hour moving means of O_2 -consumption on day n , yielded a coefficient of 0.58 ± 0.087 . This rather high and unquestionably real correlation indicated that during this 60-day period, the potatoes must have been overwhelmingly of one sign in their correlation with the unknown external factor, namely positive, and, furthermore, this unknown effective force must have retained a high correlation with the morning barometric pressure change during this period. In no other lag or lead relationship, except for smaller and obviously explicable real correlations on days $n+1$ and $n-1$, did there appear from inspection to be correlations significantly different from zero.

$n - 2$ (0.0437 ± 0.059) and day $n - 4$ (0.0622 ± 0.057) of pressure change. The next highest correlation with hourly barometric pressure, and also highly significantly different from zero, was seen for barostat No. 5 for the same period. Barostats No. 1, 2 and 4 showed no comparable correlation. These results, together, clearly suggested that the failures to obtain correlations at some times with barometric pressure were not due to a lack of capacity of the potatoes to respond to a pressure-correlated external factor, but rather due to a failure to observe the response due to a mutual cancelling of opposite signs of response.

Finally, when all the 2976 available hours of data obtained from May 1 through June 8 were correlated with the hourly differences from the daily means for barometric pressure, a value of -0.0697 ± 0.01825 (Fig. 6) was obtained. This value is obviously not zero though very small chiefly because it must be the residual after cancellation of the frequent changes of sign of response to the barometric-pressure-correlated variable. The calculated regressional relationship between the rate of O_2 -consumption, and the pressure, for all data, ignoring inversions, shows the rate of O_2 -consumption to increase from about 6.8 to about 8.2 arbitrary units, or about

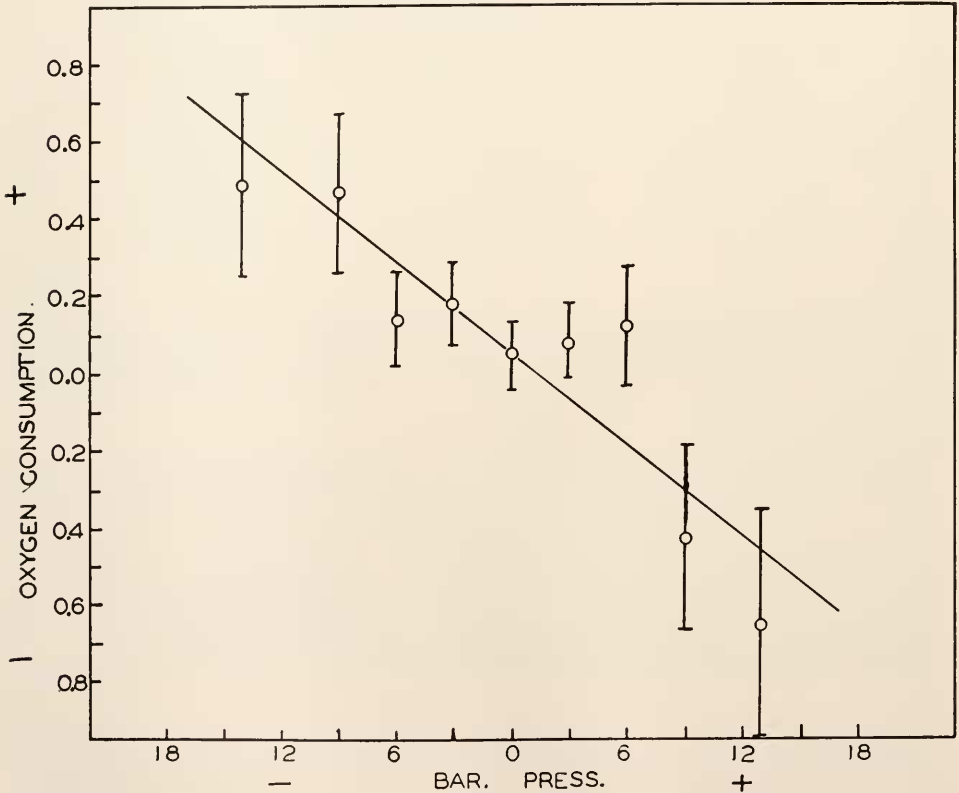


FIGURE 6. The relationship between the deviations from the daily mean of O_2 -consumption of five groups of potatoes in barostats between May 1 and June 8, 1955, and the concurrent deviations from the daily mean of barometric pressure ($P < 0.002$).

21%, as the pressure ranges from 0.15 inches Hg above to about 0.15 inch Hg below the daily mean, a moderately large range in the normal pressure changes for a single day.

DISCUSSION

It is quite clear from the preceding account that even when pressure, in addition to light, temperature, humidity and certain other factors are kept constant, there are still substantial fluctuations in O_2 -consumption in potatoes. Pressure changes are obviously not the factor responsible. This was strongly suggested in earlier studies (Brown, Freeland and Ralph, 1955) inasmuch as the day-by-day drift in O_2 -consumption at 4-7 P.M. appeared to parallel the day-by-day drift in mean barometric pressure, but apparently tending to lead the changes by one or two days.

One might suspect that possibly pressure changes were in some manner responsible for the general inversion of the 1955 cycles relative to the 1954 ones or for the hour-by-hour correlations with pressure change obtained in 1954. That this was not so, has been ascertained through other experiments using the fiddler crab and *Fucus* in our laboratory during the summer of 1955. These results, to be published elsewhere, show the cycles of these species also to be essentially inverted relative to comparable ones obtained in 1954 and similarly to show no longer the over-all hourly correlation with concurrent pressure changes. With the fiddler crab, however, parallel studies were made under conditions of fluctuating pressure exactly as was done in 1954; the cycles were, like those of the crabs in the barostats, similarly inverted relative to the 1954 ones, and similarly showed no significant over-all hourly correlation with pressure changes. There appears to be only one tenable hypothesis at this time concerning these inversions between the two years, namely, that some significant difference occurred between 1954 and 1955 with whatever fluctuating external factors are responsible for determining the form of the mean biological cycles. All organisms studied in 1955 also appeared to exhibit in some degree, the phenomenon of phase inversions relative to other individuals of the same species being studied concurrently. Whatever the mechanism, it must include to some extent the organism as a biologically responding system. It is possible that various individuals possess different thresholds for some factor which is itself responsible for the changes in sign of response to the external fluctuating factor.

The existence of the inversions which have been described in this report may lead one to question whether these fluctuations in metabolism are strictly rhythmic. Fluctuations in barometric pressure show both solar and lunar tides, but these are, in temperate zones, in good measure obscured by relatively huge climatic fluctuations. The general form of the solar tidal fluctuations may be made evident, however, through the averaging of two to five days of data. This, the potatoes seem also able to accomplish, judging from the results of this study. Hence they are apparently able to exhibit daily and lunar-day cycles of fluctuation, even though these are superimposed upon a much more randomly fluctuating background. But with the more or less erratic sign changes in 1955, the metabolism itself displays a true rhythm only for certain non-inverting components of the solar- and lunar-day cycles. The forms of the cycles are subject to the same fluctuations as are the mean daily pressure cycles. There is always the possibility, however, that the fluctuating factors which produce these responses in organisms, possess some sharper frequency-

determining component than the pressure cycles, from which the organism can obtain an adequate pacemaking signal to regulate their own internal clocks. But just as the large-amplitude random fluctuations in atmospheric pressure make it very difficult to characterize the tides therein, so may externally induced large random fluctuations in the organism tend to obscure a more precise extant organismic metabolic cyclicality.

One should always bear in mind that the metabolic cycles which were the object of this study are not as regularly rhythmic as are numerous overt organismic cycles such as color-change or motor activity in many animals. These latter must be mediated by an internal clock of the same precision as must be postulated to be functioning in these potatoes to integrate the effect of an external stimulus recurring at the same hours of the day over a few days. Such a clock is clearly needed to permit the organism to become an appropriate harmonic analyzer. Such a clock may also act as a buffer to filter out to some extent the large random elements of fluctuation in the external environment. The organism tends to retain for a time and repeat on a 24-hour cyclic basis an environmentally induced pattern of fluctuation.

It is considered highly probable that the responses to the still unknown external factor which are proven to occur by these and earlier experiments in some manner regulate the frequencies of the endogenous clocks.

SUMMARY

1. Fluctuations in O_2 -consumption in the potato under constant conditions, including pressure, were observed. The average solar-day cycle was determined and this was found to have in large measure a form which was the mirror image of that obtained during the same months of the preceding year.

2. The average lunar-day cycle was also determined and this, too, was in its principal features an inversion of that found for the same period of the preceding year.

3. A study of the patterns of daily variation for the 154 complete days of data revealed that all the patterns of fluctuation on any given day tended to exhibit a generic similarity to one another, tending to exhibit either parallel, or mirror image fluctuations, and of the same general amplitude.

4. All the daily patterns could be divided in two groups. One group (99 days) called the positive one (since its later discovered correlation with barometric pressure change was positive) possessed a maximum about 6 A.M. and a minimum about 9 A.M.; the negative group (55 days) tended to show the mirror image of this form in the daily period 4 to 11 A.M.

5. The deviation in rate of O_2 -consumption from the daily mean for the positive group for the 4-7 A.M. period was found to show a positive correlation with the algebraic sum of the rates of change in barometric pressure during the three preceding 2-4 A.M. periods; the negative group, on the other hand, showed a negative correlation for the comparable relationship.

6. A correlation between the deviation from the daily mean of O_2 -consumption at the 4-7 A.M. period and the algebraic sum of the pressure changes for the three preceding 2-4 A.M. periods was found for all 154 barostat-days if one ignored the sign of the deviation in rate of O_2 -consumption, proving true an earlier hypothesis

that the sign of the response of the organism to an external pressure-correlated factor changed from time to time.

7. The deviation from the daily mean of O_2 -consumption for the 4-7 P.M. period showed a negative correlation with the algebraic sum of the rates of barometric pressure change for the preceding three 2-4 P.M. periods, and the 10-1, midnight deviation, was correlated with the three-day pressure-change from 8-12 P.M.

8. Since it was demonstrated that the correlations were not with single days of an external factor, nor with any averaged three-day periods other than the three immediately preceding daily periods, it was evident that the potatoes were deriving an essential element of the form of their daily fluctuation from a response to an external factor which, since pressure-correlated, clearly possessed average solar-day cycles.

9. The external factor appears to determine in the daily fluctuation of O_2 -consumption the amplitude of a morning oscillation (or its mirror image) with about a six-hour period, the height of the late-afternoon maximum, and probably also the extent of the midnight reduction in rate.

10. Possible relationships of the exogenous to endogenous cycles are discussed briefly with reference to the problem of biological clocks.

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